Photoprotection of green leaves by zeaxanthin, a two-channel process

The green plants have developed the skill to not only harvest light with great efficiency, but also to dispose of the excess energy with safety. The excess light energy can cause damage to the photosynthetic apparatus of green leaves through production of singlet oxygen and other harmful oxidative species. The photosystem-II (PSII) of the thylakoid membrane of the chloroplast is the most vulnerable site of damage, obviously, due to the higher oxidizing potential of the special pigment P680 located at the reaction centre (RCII) of the photosystem. Such a high potential (estimated to be 1.17 V) is required for extraction of electrons by cleavage of water that takes place at the water-oxidizing complex. The RCII is supplemented with a group of light-harvesting antenna proteins (CP47, CP43 and LHCII) containing chlorophylls (Chl) and carotenoids. The carotenoids (carotenes and xanthophylls), besides acting as accessory pigments, are associated with photoprotection. One of the important mechanisms of photoprotection in higher plants is the dissipation of excess light energy through the xanthophyll cycle, with the formation of zeaxanthin from violaxanthin[1,2]. At higher irradiances, a pH gradient develops across the thylakoid membrane, which triggers the conversion of diepoxide violaxanthin (number of conjugated double bonds, $n = 9$) to zeaxanthin ($n = 11$) through the monoepoxide antheraxanthin ($n = 10$; Figure 1). The de-epoxidized xanthophyll, zeaxanthin, is known to facilitate dissipation of excess light energy by thermal relaxation.

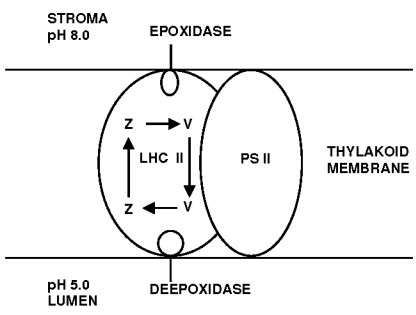

Figure 1. Highlight-induced operation of xanthophyll cycle with conversion of violaxanthin to zeaxanthin by activity of de-epoxidase.

The three major competitive routes for quenching of light energy in the thylakoid are photochemistry, thermal dissipation and emission of light, primarily by Chl*a* fluorescence. Therefore, an increase in rate of thermal relaxation is reflected in a decrease in fluorescence, called non-photochemical quenching of Chl*a* fluorescence (NPQ). In spite of the accumulation of vast literature on energy quenching (qE) by the xanthophyll cycle, its exact location and molecular mechanism remain obscure. Moreover, the *modus operandi* of zeaxanthin in qE is still controversial. A group of workers suggest that zeaxanthin can directly quench the singlet Chl energy due to its lower S_1 energy level[3]. On the other hand, Ruban *et al.*[4] describe zeaxanthin as a modulator of qE. Their experiments, of course with isolated LHCII, reveal that violaxanthin causes disaggregation of LHCs favouring light harvesting and its de-epoxidation to zeaxanthin triggers a quenched state by LHC aggregation. Experiments with mutants of *Chlamydomonas* suggest that lutein, in addition to zeaxanthin and antheraxanthin, contributes to NPQ[5]. Although most of the studies accentuate the role of zeaxanthin in NPQ, the antioxidant effect of the xanthophyll cycle, with zeaxanthin as a better quencher of singlet oxygen, has also been demonstrated[6]. In a recent paper, Li *et al.*[7] working with *Arabidopsis* mutant, emphasize that a 22-kDa PsbS protein has an exclusive role in, and is essential for photoprotective energy dissipation. In the same paper, they conclude that the appearance of LHC proteins involved in photoprotection precedes those involved in light harvesting during the evolution of oxygenic photosynthesis.

In the background of the diverse experimental results not leading to a consensus, we propose a theoretical model explaining the possible roles of zeaxanthin along with the other carotenoids during photoprotection. Even though the protonation and zeaxanthin-induced formation of a quenching complex with fluorescence lifetime of 0.4 ns has been shown[8], the details of the mechanism of formation of such a complex have been worked out in this model. The quenching complex involving Chl*a*, zeaxanthin and LHC protein (CP) is formed only at a low lumen pH (pH < 5), possibly at the interface of RCII core and peripheral LHCII[8]. The glutamic acid (Glu) on the lumenal side of helix C of LHC, equivalent to Glu-131 of LHCIIb of pea, is the proposed site for binding of zeaxanthin[9,10]. In the unquenched condition (pH > 5), the negative carboxylate ion in Glu of CP may form a ligand to Mg^{2+} of Chl*a* (Figure 2). The negative charge is delocalized over the two oxygens due to resonance and thus prevents the binding of zeaxanthin. When protonation occurs in the quenched condition, the carboxylate ion loses its charge and becomes neutral. The C=O group of carboxylic acid now becomes the potential group for ligand formation due to higher negative charge on O by π-bond polarization, compared to –OH group. This leads to a change in side-chain conformation of Glu and –OH is oriented in a direction to form H-bond with –OH group of zeaxanthin. The large molecular complex of Chl–zeaxanthin–CP acts as a sink for thermal dissipation of excess energy.

The model extends further to involve the novel idea of functional integration of β-carotenes present in the reaction centre and the quenching complex. Under photoinhibitory conditions $^1O_2$ is generated at RCII by ^{3}P680. This excitation energy is taken up by β-carotene at RCII ($\beta\text{-car}_{RC}$) and there is a possibility of this energy being channelled to the quenching complex via the β-carotene molecules

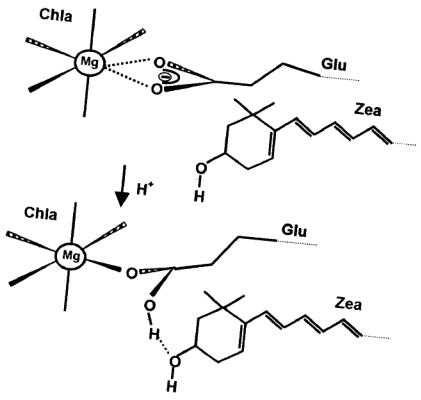

Figure 2. Formation of molecular quenching complex at acidic lumen pH induced by high light stress.

present in core antenna like CP43, CP47 (β-car$_{CP}$). Here, it is surmised that two neighbouring carotenoids are within the collisional distance. There is no experimental information available till date regarding distances between carotenoids in PSII. However, keeping in view the topology of D1, D2, CP43, CP47, CP26, and CP29 (refs 11, 12), end-to-end arrangement of the β-carotenes to achieve the required proximity for the transfer of triplet energy from RCII to zeaxanthin at the quenching complex (Zea$_{QC}$) by Dexter exchange mechanism[13] is possible, as depicted below:

$$\beta\text{-car}_{RC}(T_1) + \beta\text{-car}_{CP}(S_0) \rightarrow \beta\text{-car}_{RC}(S_0) + \beta\text{-car}_{CP}(T_1),$$

$$\beta\text{-car}_{CP}(T_1) + \text{Zea}_{QC}(S_0) \rightarrow \beta\text{-car}_{CP}(S_0) + \text{Zea}_{QC}(T_1),$$

$$\text{Zea}_{QC}(T_1) \xrightarrow{\text{Thermal relaxation}} \text{Zea}_{QC}(S_0) + \text{Heat},$$

where S_0 and T_1 stand for ground state singlet and first excited state triplet, respectively.

The inability of violaxanthin to form a complex is explained by the following reasons: (1) The epoxide group of violaxanthin provides a steric hindrance to form a complex with Glu and hence the physical proximity between Chl and violaxanthin is less. (2) Violaxanthin, with lesser number of conjugated double bonds, has its S_1 energy level much higher than that of Chl*a* to permit an excitonic interaction. On the other hand, de-epoxidation of violaxanthin to zeaxanthin removes these hindrances.

The proposed model takes into consideration an interaction of β-carotenes and zeaxanthin and also suggests the formation of an array of carotenoids that function in a sequence during photoprotection. Above all, zeaxanthin is involved not only in NPQ, but also in quenching of triplet energy migrating from RCII. Thus, for photoprotection, zeaxanthin may operate in two different channels, corroborating the views of Havaux *et al.*[6]. The initiation of the triplet channel, possibly takes place at a later stage of severe photo-inhibition. Here, the β-carotenes in CP43, CP47 are assigned with the role of mediating triplet energy transfer, thereby resolving the question raised with regard to their mechanism of action in quenching singlet oxygen produced at RCII[14].

The present model can be extrapolated to understand the undergoing photophysical and structural changes with formation of a quenching complex, where zeaxanthin dissipates energy in two different channels, quenching singlet Chl in one channel and triplet β-carotene in the other.

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Silver nitrate influences *in vitro* shoot multiplication and root formation in *Vanilla planifolia* Andr.

Vanilla is an important spice crop, and offers excellent scope for cultivation in the tropical high-rainfall regions in southern India. There is a growing demand for natural vanilla flavour in the global trade[1]. Vanilla essence, vanillin, extracted from the cured beans is widely used for flavouring cakes, sweets, chocolates, ice creams, beverages, in cosmetics and perfumery industries[1]. Vanilla, a vine orchid, is generally propagated by stem-cuttings. However, this method of propagation is rather slow, labour-intensive and time-consuming. Obtaining the stem-cuttings from the mother plants causes set-back to their growth and yield[2,3]. *In vitro* multiplication of *Vanilla planifolia* has been reported through the callus culture[4,5], protocorms, root tips[6] and axillary-bud explants[7]. The use of ethylene inhibitors like silver nitrate is found to be beneficial for shoot multiplication, rooting and in plant tissue culture studies[8]. Our earlier reports indicate the efficiency of silver nitrate in *in vitro* shoot multiplication of chicory plants[9]. In this report we provide an improved